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Antipyretic Activity of Hydroalcoholic Extract of *Dactylorhiza Hatagirea* Roots & *Lavandula Stoechas* Flowers on Brewer's Yeast Induced Pyrexia in Wistar Rats

Brijesh Sirohi*, Rakesh Sagar

Vedica College of pharmacy, RKDF University, Gandhi Nagar, Bhopal, M.P. (India)

ABSTRACT

Medicinal plants are the part and parcel of human society to combat against different diseases from the dawn of human civilization. According to World Health Organization, approximately 85% population of the developing countries are facing difficulties to afford synthetic drugs and are relying on traditional medicines mainly of plant origin in order to maintain their primary health care needs. Plants are being used in various disorders. The present pharmacological investigation was undertaken to study the anti-pyretic activity of hydroalcoholic extract of *D. hatagirea* roots (HEDH) & *L. stoechas* flowers (HELS) in albino rats against yeast induced pyrexia. Hydroalcoholic extract of *D. hatagirea* roots & *L. stoechas* flowers (5, 50, 300 and 2000 mg/ kg) orally to observe acute toxicity, and observed for 14 days. Eight groups of six animals were used for the experiment. The yeast induced pyrexia method was standardized first by injecting 15% yeast suspension (s.c) followed by recording the rectal temperature at regular intervals. Then the evaluation of anti-pyretic activity of hydroalcoholic extract of *D. hatagirea* roots & *L. stoechas* flowers (100, 200mg/kg & 300mg/kg) was carried out by using this standard procedure. The extract of *D. hatagirea* & *L. stoechas* plant showed a significant ($P<0.01$) dose dependent antipyretic effect in yeast induced elevation of body temperature in experimental rats. The data generated during study shows that *D. hatagirea* roots & *L. stoechas* flowers having significant anti-pyretic activity.

Keywords: *Dactylorhiza Hatagirea, Lavandula Stoechas,* Anti-pyretic activity, Yeast induced pyrexia

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***Address for Correspondence:**

Brijesh Sirohi, Vedica College of pharmacy, RKDF University, Gandhi nagar, Bhopal, M.P. (India)

INTRODUCTION

The hypothalamus regulates body temperature with a delicate balance between heat production and heat loss through the set-point control. Infection, tissue damage, inflammation, graft rejection, malignancy and other disease may elevate the set point to induce fever[1]. Fever is a complex physiologic response which triggered by abnormalities in the brain, toxic substances that affect temperature regulation, bacterial infections, brain tumors, and dehydration. Elevation of the body temperature occurs when the concentration of prostaglandin E_2 (PGE_2) increases within parts of the brain. The mechanism of antipyretic drugs is inhibiting the cycloxygenase (COX) activity and consequently reducing the levels of PGE_2. Synthetic antipyretic drugs have side effects[2]. Therefore, it is worthed to searching herbal medicines that are equally efficacious and comparatively side effects free, as substitutes for synthetic drugs, in recent years herbal medicine is a major component in all traditional medicine systems, and a common element in Siddha, Ayurvedic, Homeopathic, Naturopathic, Traditional Chinese medicine, and Native American medicine. Considerable efforts have been directed towards the development of natural products from various plant sources[3] [3]. *Dactylorhiza hatagirea* (D.Don) Soo belongs to the family Orchidaceae. *D. hatagirea* is a Himalayan endemic medicinal orchid which is found in Hindu Kush Himalaya range. Its occurrence is sub-alpine and alpine zones from 2800-4200 m above from sea level[4]. Other than Nepal Himalayas, it occurs in the same altitudinal ranges of India, Pakistan, Bhutan and China also. It is a terrestrial, erect herb, up to 60 cm high, with palmately divided tuberoids. Leaves are broadly lanceolate or oblong-lingulate or elliptic. Flowers purplish-lilac, rose or rarely white, in many-flowered densely cylindric inflorescence[5]. The special character of this plant is that, it remains erect in excessive snowfall. Tubers are sweet, cooling, emollient, astringent, aphrodisiac, demulcent, rejuvenating and nervine tonic. They are useful in diabetes, hemiplegia, dysentery, phthisis, chronic diarrhoea, seminal weakness, neurasthenia, cerebropathy, emaciation and general debility. A decoction of tuber is given in colic pain. Powder is used to relieve

fever; it is sprinkled over wounds to check bleeding. Root is also used in urinary troubles; also used as farinaceous food[6]. Tubers contain a glycoside, a bitter substance, starch, mucilage, albumen, a trace of volatile oil and ash[7]. Chemically, dactylorhins A-E, dactyloses A and B and lipids etc. are found as major constituents. Lavandula genus is an important member of Labiatae (Lamiaceae) family. Lavandula species are widely distributed in the Mediterranean region and cultivated in France, Spain and Italy. In Turkey mainly two species, *L. angustifolia* and *L. stoechas* and their subspecies and hybrid forms grow wildly or they are cultivated[8,9]. *L. stoechas* is known as French lavender[10] and it is used by people for various diseases of central nervous system (epilepsy and migraine), treatment of wounds, reduce to blood sugar. It is also used as antispasmodic, antiseptic, antimicrobial, sedative, diuretic and analgesic agents[11-13]. Lavender has also positive effects on urinary infections, cardiac diseases and eczema[12]. Furthermore, nowadays the lavender species are extensively cultivated throughout the world in order to be commonly employed in perfumery and cosmetic industry[12-14], where especially their oil extracted is used. Lavender essential oil is used in aromatherapy and in the flavouring and fragrance industry[15,16]. Lavender has been extensively studied from the phytochemical point of view, limited studies being devoted to pharmacological aspects[17]. For medicinal purposes its aerial parts are used. The aerial parts contain oleanolic acid, ursolic acid, vergatic acid, *β*-sitosterol, *α*-amyrin, *α*-amyrin acetate, lupeol, erythrodiol and flavonoids, acacetin and vitexin[18], as well as two longipinane derivatives, longipin-2- ene-7*β*,9*α*-diol-1-one and longipin-2-ene-7*β*,9*α*-diol-1-one-monoacetate[19]. Recent studies also suggested that Lavandula species have antioxidant and local anaesthetic activity[20]. Since the antipyretic activity effect of *D. hatagirea* roots & *L. stoechas* flowers has been experimentally not confirmed. So the present study has been carried out to evaluate and compare the in vivo antipyretic activity of the hydroalcoholic extract of *D. hatagirea* roots & *L. stoechas* flowers by yeast induced pyrexia method.

MATERIALS AND METHODS

Plant material

The roots of plant *D. hatagirea* and aerial part of *L. stoechas* were collected from local area of Bhopal (M.P), India in the month of Jan 2017. The taxonomical identification and authentication of the plant material was done by Dr. Zia Ul Hasan, Department of Botany, SAFIA College Bhopal (M.P). The specimens of voucher have been submitted and preserved in the herbarium of SAFIA College Bhopal (M.P).

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Preparation of extract

Defatting of plant material

Powdered material of *L. stoechas* and *D. hatagirea* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.

Extraction by hot continuous percolation process

100 gm of *L. stoechas* and *D. hatagirea* dried material were exhaustively extracted with 80% ethanol (Hydroalcoholic) using hot continuous percolation for 24 hrs. Appearance of colorless solvent in the siphon tube was taken as the end point of extraction. The extracts were concentrated to ¾ of its original volume by distillation. The concentrated extracts were taken in a china dish and evaporated on a thermostat controlled water bath till it forms a thick paste and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[21].

Experimental animals

Swiss albino male mice (20-25 g) were group housed (n= 6) under a standard 12 hr light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55-65%). Mice received standard rodent chow and water *ad libitum*. Animas were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00hr. Separate group (n=6) of mice was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute toxicity studies

Acute toxicity studies were conducted on Swiss albino mice as per the Organization for Economic Co-operation and Development (OECD) 423 guidelines[22]. The hydroalcoholic extract of both plants at doses of 5, 50, 300 and 2000 mg/ kg body weight were administered to four groups of rats (n = 6) after overnight fasting. The animals were observed twice on the day of the dosing and once daily thereafter for 14 days. Animals were observed daily for mortality and for gross changes in activity and behavioral pattern. They were also observed for the presence of tremors, convulsions, salivation, diarrhea and lethargy. The hydroalcoholic extract of *D. hatagirea* roots & *L. stoechas* flowers was found to be non-toxic up to a dose of 2000 mg / kg and did not cause death, therefore it was considered to be safe. Hence, one-tenth of this dose, that is, 200 mg/kg body weight and half of the one-tenth dose, that is, 100 mg/kg, as also 3times of this, that is, 300mg/kg, were used for the elucidation of anti-pyretic activity.

Brewer's yeast-induced pyrexia

Before experimentation rectal temperature of mice were recorded by inserting a well lubricated bulb of a thermometer in the rectum. Hyperpyrexia was induced in mice by subcutaneous injection of 10 ml/kg b.w. of a 15% aqueous suspension of brewer's yeast in the back below the nape of the mice. Pre-drug control temperatures were taken at 24 h after the yeast injection to determine the pyretic response of yeast. HELS (100, 200 and 300 mg/kg, p.o.), HEDH (100, 200 and 300 mg/kg, p.o.) and aspirin (100 mg/kg body weight) served as the reference drug given orally 24 h after the yeast injection. The temperatures were recorded at 1-4 h after the drug treatment[23, 24]. The followings are group distribution,

Group I – Control mice (Normal saline 5 ml/kg)

Group II – Standard (Aspirin 100 mg/kg/ p.o.)

Group III – HELS 100 mg / kg, p.o.

Group IV – HELS 200 mg / kg, p.o.

Group V – HELS 300 mg / kg, p.o.

Group VI – HEDH 100 mg / kg, p.o.

Group VII – HEDH 200 mg / kg, p.o.

Group VIII – HEDH 300 mg / kg, p.o.

Statistical analysis

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test and P<0.05 were considered to be statistically significant.

RESULTS AND DISCUSSIONS

The effect of hydroalcoholic extract of HELS and HEDH plant on yeast induced pyrexia has been shown in Table 1 and 2. Treatment with extracts at dose of 100, 200 mg/kg and 300 mg/kg body weight and aspirin at dose of, 100mg/kg decreased body temperature of yeast induced mice in a dose-dependent manner. The antipyretic effect started as from the first hour and the effect was maintained for 4 h, after administration of the extract. The results obtained from both standards and extracts treated groups were compared with the control group. A significant reduction in the yeast elevated rectal temperature was observed in the test drug.

Fever can be induced in experimental animals by intravenous or subcutaneous injection of pyrogens. To evaluate the antipyretic activity of test drugs, the most commonly employed method to induce fever involves injection of lipopolysaccharides (LPS) or brewer's yeast in rabbits or rats. Antipyretic are the agents, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates and a drug like aspirin does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature[25, 26]. Yeast induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature. The present results show that both extract possesses a significant antipyretic effect in yeast- provoked elevation of body temperature in mices and its effect is comparable to that of aspirin (standard drug). So inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of aspirin. Also, there are several mediators or multiprocesses underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyretic effect[27, 28].

Table 1 Effect of HELS and HEDH extract on Yeast-induced hyperpyrexia (before drug admin.) in mice

S.N.	Groups	Dose	Temp. Before yeast admin.	Pre-drug control, 1h before drug admin.
1.	Normal	5 mL/kg/p.o.	95.56±3.88	101.27±0.34
2.	Aspirin	100 mg/kg/ p.o.	95.43±4.48	100.17±0.30
3.	HELS-100	100 mg/kg, p.o.	96.13±5.62	100.10±0.85
4.	HELS-200	200 mg/kg, p.o.	95.57±4.35	100.07±0.29
5.	HELS-300	300 mg/kg, p.o.	95.23±4.49	100.50±0.12
6.	HEDH-100	100 mg/kg, p.o.	96.12±5.61	100.08±0.84
7.	HEDH-200	200 mg/kg, p.o.	95.56±5.34	100.05±0.28
8.	HEDH-300	300 mg/kg, p.o.	95.23±4.48	100.45±0.10

Table 2 Effect of HELS and HEDH extract on Yeast-induced hyperpyrexia (After drug admin) in mice

Groups	Dose	Temp. Before yeast admin.	Rectal temp. After drug admin. (% decrease)			
			1 h	2 h	3 h	4 h
Normal	5 ml/kg/p.o.	95.56±3.88	101.10±5.42	101.07±4.38	101.17±5.00	101.07±5.44
Aspirin	100 mg/kg/ p.o.	95.43±4.48	98.20±5.49***	96.00±5.56***	95.50±4.40***	92.47±4.42***
HELS-100	100 mg/kg, p.o.	96.13±5.62	99.57±4.57**	98.80±6.35**	97.33±5.52**	96.57±3.58**
HELS-200	200 mg/kg, p.o.	95.57±4.35	99.30±4.29**	98.57±4.29**	97.00±4.15**	96.40±5.20**
HELS-300	300 mg/kg, p.o.	95.23±4.49	99.00±5.29**	97.89±5.67***	96.17±4.44***	94.00±5.50***
HEDH-100	100 mg/kg, p.o.	96.12±5.61	99.56±6.56**	98.89±5.34**	97.32±4.52**	96.96±4.58*
HEDH-200	200 mg/kg, p.o.	95.56±5.34	99.32±5.28**	98.00±6.28***	97.12±6.14**	96.39±5.21**
HEDH-300	300 mg/kg, p.o.	95.23±4.48	99.00±4.28**	97.70±4.66***	96.16±5.43**	94.40±5.49***

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at***P<0.001, **P<0.01, *P<0.05 vs. control group respectively (One-way ANOVA followed by Tukey's post hoc test).

CONCLUSION

The present investigation it may be concluded that the hydroalcoholic extract of HELS and HEDH have antipyretic activity. In this study no attempt was made to ascertain the mechanism of the observed antipyretic activity. However, it can be suggested that it may be acting through either the peripheral or central mechanism enumerated above. It is also possible that both the mechanisms may be involved. Further, study regarding isolation and characterization of active principle responsible for antipyretic activity are under planning in our laboratory.

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